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ORIGINAL PAPER

Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion

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Abstract Twenty endophytic bacteria were isolated from the meristematic tissues of three varieties of strawberry cultivated in vitro, and further identified, by FAME profile, into the genera *Bacillus* and *Sphingopyxis*. The strains were also characterized according to indole acetic acid production, phosphate solubilization and potential for plant growth promotion. Results showed that 15 strains produced high levels of IAA and all 20 showed potential for solubilizing inorganic phosphate. Plant growth promotion evaluated under greenhouse conditions revealed the ability of the strains to enhance the root number, length and dry weight and also the leaf number, petiole length and dry weight of the aerial portion. Seven *Bacillus* spp. strains promoted root development and one strain of *Sphingopyxis* sp. promoted the development of plant shoots. The plant growth promotion showed to be correlated to IAA production and phosphate solubilization. The data also suggested that bacterial effects could potentially be harnessed to promote plant growth during seedling acclimatization in strawberry.

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Introduction

The brazilian states of São Paulo and Minas Gerais are the biggest producers of strawberries (*Fragaria ananassa*) in Latin America (Botelho 1999). Brazilian strawberry crops are based in the cultivation of plants obtained from plant tissue cultures, using somatic embryogenesis (Williams and Maheswaran 1986; Smýkal et al. 2007). This technique makes it possible to produce a great number of clones, free of pathogenic fungi and bacteria (Siragusa et al. 2007). However, the process of explant disinfection might also eliminate non-pathogenic microorganisms, which could be important in subsequent cultivation steps such as seedling acclimatization.

The feasibility of using bacterial inoculants during acclimatization, to promote plant colonization by a favorable rhizospheric and endophytic microbial community, has been considered in order to optimize the development of the plants (Khalid et al. 2004). These bacteria should possess mechanisms for one of the following functions: (i) biological control of phytopathogens, (ii) phytohormone production, or (iii) supply of plant nutrients (nitrogen or phosphate) (Christiansen-Weniger and Van Veen 1991; Roesch et al. 2007). Bacterial inoculants can be generated by endophytic bacteria that can colonize the rhizosphere before penetrating host plant tissues (Azevedo 1998; Lodewyckx et al. 2002; Andreote et al. 2006). Although endophytes can interact with plants in different ways, particularly important endophyte characteristics related to plant growth promotion are the production of auxin-like molecules (Costacurta et al. 1995; Patten and Glick 1996;

Spaepen et al. 2007) and phosphate solubilization (Barea et al. 1983; Ryan et al. 2008).

The aims of this work were firstly to identify and characterize the capacity for phosphate solubilization and IAA production found in endophytic bacteria that is associated with micropropagated strawberry seedlings, and secondly to study the ability of these strains to promote the growth of micropropagated strawberry seedlings during the acclimatization process.

Materials and methods

Plants used and isolation of endophytic bacteria

The bacterial samples used in the present work were obtained from strawberry tissue culture laboratories locate in the city of Pouso Alegre (Minas Gerais, Brazil), where plant multiplication is performed prior to cultivation in the field. Ten meristematic segments from each of three varieties of strawberry (Camarosa, Oso-Grande and Sweet Charlie) were selected and used for bacterial isolation.

Each plant sample consisted of a fragment containing the meristematic region used in the micropropagation process. Samples were surface sterilized according to a previously described methodology for endophytic bacterial isolation (Araújo et al. 2002). Briefly, tissues were subjected to serial immersions in 70% ethanol for 1 min, 2.5% sodium hypochlorite for 20 min, and 70% ethanol for 30 s, followed by three rinses in sterile deionized water. The extended time in hypochlorite (20 min) was used to enhance the disinfection of tissues surrounding the meristematic region. After surface disinfection, meristematic regions were extracted and cut into pieces of approximately 0.2 cm which were transferred to Petri dishes containing solid MS medium (Murashighe and Skoog 1962). The plates were incubated at 28°C and monitored daily, over a two week period, for bacterial colony development. After bacterial growth had occurred, colonies were purified by streaking, and isolated colonies were picked from the plates and used to inoculate 5% Trypic Soy Agar slants. Colonies were also cultured in 5% Trypic Soy Broth at 28°C for 36 h, suspended in 20% glycerol solution and stored at −70°C.

Strain identification by FAME-MIDI

In order to identify the isolated bacterial strains, bacteria were cultured on TSA and submitted for fatty acid methyl ester (FAME) analysis by gas chromatography using an automatic injector and a Flame Ionization Detector (FID) (Agilent 6850 and 7683). The output data were organized into a chromatogram and the identification report was prepared using the Microbial Identification System software (Sherlock TSBA40 library; MIDI Inc., Newark, DE, USA). The similarity of 0.70 with hits in the database were used to classify strains at species level, while lower values were considered for affiliation of isolates at higher taxonomic levels. The final results appeared to confirm the similarities found between the database and the nominated areas, enabling the strains to be identified.

Screening of endophytes for phosphate solubilization

Phosphate solubilization by the newly isolated strains was evaluated according to the methodology previously described by Mehta and Nautiyal (2001). Briefly, strains were cultivated in medium PVK (glucose 1%, $Ca_3(PO_4)_2$ 0.5%, $(NH_4)_2SO_4$, 0.05%, NaCl, 0.02%, $MgSO_4 \cdot 7H_2O$ 0.01%, KCl 0.02%, yeast extract 0.05%, $MnSO_4 \cdot H_2O$, 0.0002%, $FeSO_4 \cdot 7H_2O$, 0.0002%, agar 1.5%), where the ability to grow is associated to the capacity in using $Ca_3(PO_4)_2$ as a sole phosphate source. After the cultivation (14 days), halos were observed surrounding colonies which were able to solubilize inorganic phosphate.

The quantitative determination of phosphate solubilization was performed as previously described previously by Marinetti (1962). Strains were cultivated in liquid PVK medium, and after growing, cells were harvested (3000*g* for 5 min) and supernatant were used for colorimetric quantification of available phosphate. The staining of phosphate in the supernatant was performed using 2 ml of the supernatant in combination with 1 ml of vanadate solution (NH_4VO_3 0.25% in 35% HNO_3) and 1 ml of molibdate solution ($(NH_4)_6MO_7O_{24}$ 5% in water). After the development of a reddish color (approx. 5 min.), solutions were submitted to spectrophotometer analysis at 420 nm.

Screening of endophytes for auxin-like molecule production

The production of auxin molecules was determined by the colorimetric methodology described previously by Gordon and Weber (1951). Briefly, isolates were first screened for IAA production by cultivation in Trypic Soy Agar plates supplemented NH_4Cl_2 (10 mM) and L-tryptophan (100 µg/ml). Colonies were covered with nitrocelullose membrane and incubated for 48 h in the dark. Membranes were removed and stained with Salkovski reagent ($FeCl_3 \cdot 6H_2O$ 1.5 mM, HCl 8 M). Positive strains were verified by the development of red staining around the colonies. These strains were submitted to quantitative analysis of IAA production by growing in nutrient broth (NB) medium amended with tryptophan (100 µg/ml) in the dark. Cells were harvested by centrifugation (12,000*g* for 5 min) and the supernatant was treated with Salkovski

reagent for 15 min. The production of IAA was direct related to the absorbance measured at 530 nm. Pure indole-acetic-acid (IAA) was used in all experiment as a standard.

Plant growth promotion experiment

Micropropagated strawberry seedlings of the Oso Grande variety were used. Prior to treatment, the seedlings were transferred to plastic tubes (9 cm high and 3.5 cm in diameter) containing autoclaved substrate (EUCATEX, São Paulo, Brazil).

Endophytic strains used in the inoculation were grown in liquid Nutrient Broth for 24 h at 28°C. The cells were then harvested and bacterial cell suspensions were prepared in water (10^8 cfu/ml^{-1}). Aliquots of 100 µl from the bacterial suspensions were inoculated at the base of the plant stems, near the substrate interface, in the region called the hypocotyl. In total, 15 plants were inoculated with each strain. Fifteen control plants received inoculation with 100 µl of water only.

Plants from all treatment groups were maintained in a greenhouse for three months, at 30°C. The plants were then removed from the tubes and washed in running water. Every plant was analyzed for the following variables: root number, length and dry weight, number of leaves, petiole length and dry weight of shoots. The mean calculated values for each estimated parameter were statistically compared using standard procedures including the SAS general linear model (GLM) and least significant difference (LSD) analysis at the 5% level of probability (SAS Version 8.01, SAS Institute, Inc, Cary, NC).

Results and discussion

Isolation and identification of endophytic bacteria in micropropagated strawberry plants

Endophytic bacteria colonizing in vitro strawberry tissues were found in samples from all three varieties. However, the frequency of endophytic isolations was found to differ among plant genotypes. Meristematic regions of the Oso Grande and Camarosa varieties more frequently harbor endophytic bacteria than those of the Sweet Charlie variety (Fig. 1). Endophytic bacteria that colonize the meristematic tissues and do not cause any damage to plant development can be surveyed for their potential to improve plant development, conferring benefits to the plant and resulting in an enhanced symbiotic system.

The FAME technique was used for identification and allowed us to infer the phylogeny of 17 bacterial strains, distributed along three genera (*Bacillus*, *Sphingopyxis* and *Virgibacillus*), and comprised of two bacterial families; Bacillaceae (Bacilli), and Sphinogmonadaceae (Alphaproteobacteria) (Table 1). The 20 strains were classified into species with similarities values varying from 0.295 to 0.926 with the match found in the FAME database. FAME identification is highly reliable for similarities higher than 0.70 at species level, while lower levels can affiliate isolates to higher taxonomic groups, like genus or families (Heyrman et al. 1999). Considering that, identified strains were four strains of *Bacillus* sp., seven *Bacillus subtilis*, three *Bacillus megaterium*, one *Virgibacillus* sp. and one *Sphingopyxis* sp. The three remaining strains could not be matched to any known species by the FAME technique (Table 1). The prevalence of *Bacillus* spp. was postulated to be due to possible resistance of the bacteria to the disinfection process prior to submission of explants to tissue culture.

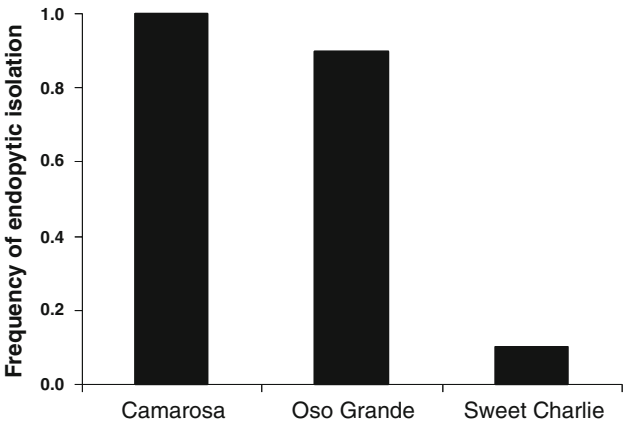

Fig. 1 Frequency of endophytic bacteria isolation from meristematic regions of different varieties of strawberry plants. The frequency was determined by the number of infected samples divided by the number of tissue fragments placed on medium plates

Phosphate solubilization and auxin production

The mechanisms by which the endophytic strains from strawberry plants could influence plant growth were investigated by assessing their capacity for phosphate solubilization and auxin production. Phosphorus, one of the main nutrients limiting plant growth, is rapidly immobilized after addition to soil as a soluble fertilizer, becoming unavailable to the plant. Therefore, bacterial activity is highly important with respect to supplying plants with phosphorus. Endophytes are known to promote plant growth by phosphate solubilization (Verma et al. 2001; Wakelin et al. 2004). Soil inoculation with phosphate-solubilizing *Bacillus* spp. can solubilize fixed soil P and applied phosphates, resulting in a better plant development and higher yields (Yadav and Dadarwal 1997; Puente et al. 2004a and b; Canbolat et al. 2006). Qualitative phosphate

Table 1 Identification of bacterial strains and assessment of their growth promotion potential in strawberry seedlings

Origin		Identification		Seedling Growth Promotion					
				Roots			Shoots		
Variety	Strains	Identity	Similarity (%)	Number	Lenght[A] (cm)	Dry weight[B] (g)	Number of petioles	n° leaves	Dry weight (g)
Camarosa	C1	*Bacillus megaterium*	0.926	5.30 abc[A]	10.65 abcd	0.039 ab	19.14 fghi	6.80 b	0.10 abc
	C4	*Bacillus subtilis*	0.789	5.00 abc	9.72 bcde	0.025 b	21.94 cdefgh	5.91 bcd	0.10 abc
	C15	*Bacillus subtilis*	0.863	**5.91 a**[B]	**12.08 a**	0.041 ab	22.33 defgh	5.41 de	0.09 abcd
	C16	*Sphingopyxis* sp.	0.307	5.50 abc	10.50 abcd	0.034 ab	**28.77 a**	**7.78 a**	**0.13 a**
	C18	*Bacillus* sp.	0.562	5.70 ab	11.30 abc	0.041 ab	21.07 efgh	6.60 bc	0.11 ab
	C19	*Bacillus* sp.	0.658	5.66 ab	8.66 de	0.035 ab	26.90 bcde	4.55 e	0.06 cd
	C20	*Bacillus* sp.	0.586	5.0 abc	8.65 e	0.027 b	18.96 ghi	5.40 de	0.06 cd
	C22	Not identified	–	4.70 bc	**12.25 a**	0.023 b	17.76 fghi	5.90 bcd	0.06 d
	C25	*Bacillus* sp.	0.295	5.5 abc	11.79 ab	0.037 ab	26.93 abcd	6.20 bcd	0.10 abc
	C30	*Bacillus subtilis*	0.767	4.45 bc	11.50 abc	0.031 b	15.73 i	5.54 cde	0.06 cd
Oso Grande	O1	Not identified	–	**5.92 a**	12.07 a	0.045 ab	26.50 abc	6.64 bc	0.10 ab
	O11	*Virgibacillus* sp.	0.507	5.23 abc	10.84 abc	0.042 ab	17.79 ih	5.92 bcd	0.09 abcd
	O13	*Bacillus megaterium*	0.721	5.18 abc	10.59 abc	0.035 ab	24.22 bcdef	6.16 bcd	0.08 bcd
	O17	*Bacillus megaterium*	0.747	4.70 bc	9.55 cde	0.028 b	28.35 ab	6.45 bcd	0.08 abcd
	O19	*Bacillus* sp.	0.619	5.36 abc	11.45 abc	0.042 ab	19.53 fghi	5.72 bcd	0.09 abcd
	O19′	*Bacillus subtilis*	0.835	5.35 abc	**12.32 a**	**0.055 a**	23.21 cdefg	5.50 cde	0.11 ab
	O27	*Bacillus subtilis*	0.913	5.54 ab	**12.59 a**	0.032 ab	19.56 fghi	5.33 de	0.06 cd
	O28	*Bacillus subtilis*	0.744	5.28 abc	**12.57 a**	0.041 ab	25.37 abcde	6.14 bcd	0.09 abcd
	O29	*Bacillus subtilis*	0.734	4.72 bc	**12.13 a**	0.039 ab	22.35 cdefgh	6.36 bcd	0.07 bcd
Sweet Charlie	S8	Not identified	–	4.80 bc	11.35 abc	0.036 ab	29.44 ab	5.66 bcd	0.09 abcd
Ct[C]				5.10 abc	9.80 bcde	0.022 b	24.60 bcde	6.36 bcd	0.08 bcd

[A] Treatments followed by a similar letter were not statistically significant ($P < 0.05$)

[B] Bold text indicates treatments with significantly higher values than controls ($P < 0.05$)

[C] Ct indicates control plants, only treated with water

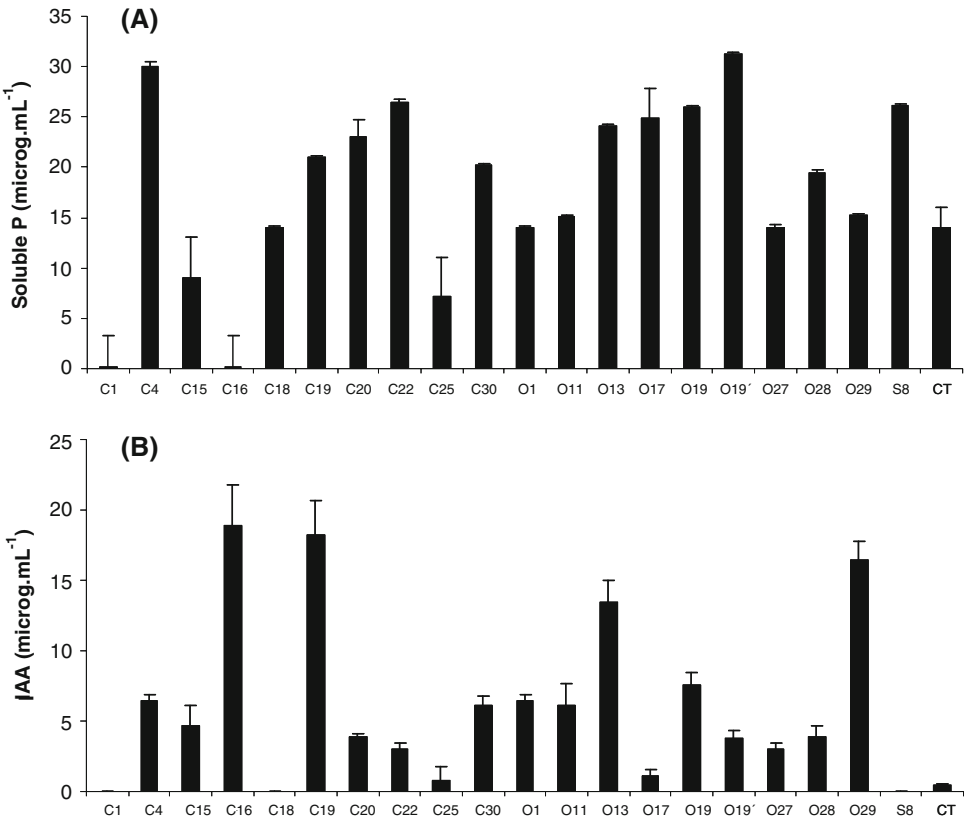

Fig. 2 Phosphate solubilization (**a**) and IAA production (**b**) observed in the endophytic bacterial strains obtained from strawberry plants. Each value represents the mean of three replicates and the error bars represent the standard deviations of the average

solubilization activity was verified for all 20 isolates. However, the isolates displayed variable efficiencies (Fig. 2). Two *B. subtilis* strains (O19'and C4) presented the best performance, while low indexes were observed for strains C1 and C16, classified as *B. megaterium* and *Sphingopyxis* sp., respectively. Although we have not done the test for compounds involved in phosphate solubilization, in *Bacillus*, the main compounds involved in the phosphate solubilization are the lactic, itaconic, isovaleric, isobutyric and acetic acids (Vazquez et al. 2000).

Endophytes can also promote plant growth by producing the phytohormone IAA (Lee et al. 2004; Mendes et al. 2007). IAA increases root size and distribution, resulting in greater nutrient absorption from the soil (Kuklinsky-Sobral et al. 2004; Li et al. 2008). When screened for auxin production, 15 isolates revealed to produce it at concentrations higher than 1 μg ml^{-1}. Among these, the highest production was observed by strains O13 (*B. megaterium*), C19 (*Bacillus* sp.), O29 (*B. subtilis*) and C16 (*Sphingopyxis* sp.) (Fig. 2). High scores for phosphate solubilizers did not match with best auxin production. For example, high production of auxin was observed in isolate C16 (*Sphingopyxis* sp.), which presented a low efficiency in solubilizing phosphate. These data indicate that plant growth promotion in the environment is not driven by a single species but is due to a composite effect of features present in several symbiotic bacteria.

Plant growth promotion by endophytic bacterial isolates

Plant growth promotion is a phenomenon driven by beneficial microorganisms which associate with plants and contribute to their better development (Christiansen-Weniger and Van Veen 1991; Roesch et al. 2007). In this study, plant growth promotion triggered by the inoculation of 20 endophytic strains in strawberry seedlings under acclimatization was evaluated by considering a number of parameters. Inoculation of the strawberry plants with the bacterial isolates resulted in enhanced plant development in many cases. However, it is important to note that, in some cases, inhibition of plant development was also observed, indicating that the endophytic state was not always maintained, and may have been dependent on the cultivation conditions.

The data from phenotypic evaluation show that from the 20 inoculated bacteria, seven promoted root growth (two increased the number of roots, six increased the root lengths and one increased root dry weights) and one promoted plant shoot development (Table 1). Promotion of root development was observed for the strains classified as *B. subtilis* C15, O19, O27, O28 and O29, and for the non-identified strain C22. In contrast, development of the aerial part of the strawberry seedlings was promoted by strain C16, identified as *Sphingopyxis* sp. (Table 1). In addition, an adaptation of strains could be inferred, due to the better results obtained

for plants promotion by strains isolated from the variety Oso Grande, which was used for the experiment. The choice of this genotype was made due to the availability of these plants in the moment of the experiment set up. Also, it should be remarked that the variety Oso Grande is the most used by the producer in the region where the work was conduced, due to the higher yield. The plant–bacteria interactions are responsive to the variations on the genotype of the involved species (Salvaudon and Shykoff 2008; Kuklinsky-Sobral et al. 2004).

Tracing the relationships between the obtained data in vitro in analyses for phosphate solubilization and auxin production with plant growth promotion observed in the greenhouse, correlations were found with both features found in isolates. Two strains with high IAA production, C16 and O29, and one strain, with high value for phosphate solubilization, O19', promoted the plant development when inoculated in plants seedlings (Table 1).

The present results are consistent with the possibility that a single ecological function can be shared by different bacterial species. For example, IAA production was observed in two isolates, C16 and O29, which were identified as distinct species *Sphingopyxis* sp. and *B. subtilis*, respectively. Meanwhile, production of auxin by different strains of bacteria could promote the development of different parts of the host plant; roots (strain O29) and shoots (strain C16). The suggestion that there may be co-colonization resulting in complementary effects on plant development by strains C16 and O29, still remains to be explored.

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References

Andreote FD, Lacava PT, Gai CS, Araújo WL, Maccheroni W Jr, Overbeek LSV, Elsas JDV, Azevedo JL (2006) Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella Fastidiosa*. Can J Microbiol 52:419–426. doi:10.1139/W05-142

Araújo WL, Marcon J, Maccheroni W Jr, Van Elsas JD, Van Vuurde JWL, Azevedo JL (2002) Diversity of endophytic bacterial population and interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914. doi:10.1128/AEM.68.10.4906-4914.2002

Azevedo JL (1998) Biodiversidade microbiana e potencial biotecnológico. In: Melo IS, Azevedo JL (eds) Ecologia microbiana, 1st edn. Jaguariuna, EMBRAPA-CNPMA v.1, pp 445–461

Barea JM, Azcón-Aguillar C, Azcón R (1983) Interactions between phosphate solubilizing bacteria and VA mycorrhiza to improve plant utilization of rock phosphate in non acidic soil. In: 3rd International congress on phosphorus coumpounds, pp 127–144

Botelho JS (1999) A situação da cultura do morangueiro no Estado de Minas-Gerais. In: Duarte Filho J (ed) Morango: tecnologia de produção e processamento, 1st edn. Caldas, Minas Gerais, pp 125–128

Christiansen-Weniger C, Van Veen JA (1991) NH^{4+}-excreting *Azospirillum brasilense* mutants enhance the nitrogen supply of a wheat host. Appl Environ Microbiol 57:3006–3012

Costacurta A, Keijers V, Vanderleyden J (1995) Synthesis of phytormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18. doi:10.3109/10408419509113531

Canbolat MY, Bilen S, Cakmakci R, Şahin F, Aydın A (2006) Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fertil Soils 42:350–357. doi:10.1007/s00374-005-0034-9

Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. Plant Physiol 26:192–195

Heyrman J, Mergaert J, Denys R, Swings J (1999) The use of fatty acid methyl ester analysis (FAME) for the identification of heterotrophic bacteria present on three mural paintings showing severe damage by microorganisms. FEMS Microbiol Lett 181:55–62. doi:10.1111/j.1574-6968.1999.tb08826.x

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96:473–480. doi:10.1046/j.1365-2672.2003.02161.x

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251. doi:10.1111/j.1462-2920.2004.00658.x

Lee S, Flores-Encarnacion M, Contreras-Zentella M, Garcia-Flores L, Escamilla JE, Kennedy C (2004) Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in cytochrome C biogenesis genes. J Bacteriol 186:5384–5391. doi:10.1128/JB.186.16.5384-5391.2004

Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40:238–246. doi:10.1016/j.soilbio.2007.08.014

Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mesgeay M, Van Der Lelie D (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21:583–606. doi:10.1080/0735-260291044377

Marinetti GV (1962) Chromatographic separation, identification and analysis of phosphatides. J Lipid Res 3:1–20

Mehta S, Nautiyal CS (2001) An efficient method for qualitative screening of phosphate-solubilizing bacteria. Curr Microbiol 43:51–56. doi:10.1007/s002840010259

Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JM (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl Environ Microbiol 73:7259–7267. doi:10.1128/AEM.01222-07

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Puente ME, Bashan Y, Li CY, Lebsky VK (2004a) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol 6:629–642. doi:10.1055/s-2004-821100

Puente ME, Li CY, Bashan Y (2004b) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II.

Growth promotion of cactus seedlings. Plant Biol 6:643–650. doi:10.1055/s-2004-821101

Roesch LFW, Quadros PD, Camargo FAO, Triplett EW (2007) Screening of diazotrophic bacteria *Azopirillum* spp. for nitrogen fixation and auxin production in multiple field sites in southern Brazil. World J Microbiol Biotechnol 23:1377–1383. doi:10.1007/s11274-007-9376-9

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. doi:10.1111/j.1574-6968.2007.00918.x

Salvaudon LGT, Shykoff JA (2008) Genetic diversity in natural populations: a fundamental component of plant–microbe interactions. Curr Opin Plant Biol 11:135–143. doi:10.1016/j.pbi.2008.02.002

Siragusa M, Carra A, Salvia L, Puglia AM, De Pasquale F, Carimi F (2007) Genetic instability in calamondin (*Citrus madurensis* Lour) plants derived from somatic embryogenesis induced by diphenylurea derivatives. Plant Cell Rep 26:1289–1296. doi:10.1007/s00299-007-0326-7

Smýkal P, Valledor L, Rodríguez R, Griga M (2007) Assessment of genetic and epigenetic stability in long-term in vitro shoot culture of pea (*Pisum sativum* L.). Plant Cell Rep 26:1985–1998. doi:10.1007/s00299-007-0413-9

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. doi:10.1111/j.1574-6976.2007.00072.x

Vazquez P, Holguin G, Puente M, Lopez E, Cortes A, Bashan Y (2000) Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi arid coastal lagoon. Biol Fertil Soils 30:460–468. doi:10.1007/s003740050024

Verma SC, Ladha JK, Tripathi AK (2001) Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J Biotechnol 91:127–141. doi:10.1016/S0168-1656(01)00333-9

Yadav KS, Dadarwal KR (1997) Phosphate solubilization and mobilization through soil microorganisms. In: Dadarwal KR (ed) Biotechnological approaches in soil microorganisms for sustainable crop production. Scientific Publishers, Jodhpur, pp 293–308

Wakelin S, Warren R, Harvey P, Ryder M (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol Fertil Soils 40:36–43. doi:10.1007/s00374-004-0750-6

Williams ES, Maheswaran B (1986) Somatic embryogenesis: factors influencing coordinated behavior of cell as an embryogenic group. Ann Bot (Lond) 57:443–462